

POLAROGRAPHIC ACTIVITY AND ELECTROLYTIC REDUCTION OF FERREDOXIN

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1. Introduction

The structure and function of ferredoxin have been the subject of intensive investigation. Its physiological participation in electron transfer, coupled with our interest in polarography as a technique for studying redox systems prompted an examination for ferredoxin polarographic activity. In this communication we report that ferredoxin does give a characteristic polarographic signal, that this is associated with the biological function of the protein and that electrolytic reduction may offer an advantageous means of preparing reduced ferredoxin. To our knowledge no studies of the analytical or preparative electroreduction of ferredoxin have previously been reported.

2. Experimental

Ferredoxin was purified from *Clostridium pasteurianum* by the method described, and to the purity indicated, by Mortenson [1]. Polarographic measurements were made with a Radiometer PO4 recording polarograph using a reaction vessel and procedure as previously described [2]. The dropping mercury electrode was employed, together with the saturated calomel reference electrode (S.C.E.). The polarograph was used at high sensitivity, in the range 0.1–0.3 μ A full-scale deflection, at which sensitivity polarograms of buffer solutions themselves have a high slope. To overcome this, condenser-current compensation was

applied, usually to the extent of 0.2 μ A/V. Electrolytic reduction was carried out by the method described by Cecil and Weitzman [3] or a modification thereof as follows. It was desirable to carry out polarographic measurements and electroreduction in the same apparatus, thereby obviating the need to transfer material from one vessel to another, with consequent risk of some oxidation of the labile reduced ferredoxin. This was achieved by using a polarographic cell as previously described [2] modified to contain a platinum wire fused through the bottom and making contact with a small pool of mercury in the cell. A small magnetic flea floated on the surface of the mercury and, during electroreduction, was rotated to stir the mercury surface. By this means it was possible to make analytical polarographic measurements by applying potentials between the dropping mercury electrode and reference calomel electrode, or to carry out preparative electroreduction by applying a potential (from an external battery or the polarograph itself) between the stirred mercury pool cathode and the calomel anode. The apparatus could readily be switched from one mode of operation to the other.

3. Results and discussion

C. pasteurianum ferredoxin was found to give a well-defined polarographic reduction wave, as shown in fig. 1. The half-wave potential at pH 7 was –570 mV (versus the S.C.E.), equivalent to –325 mV

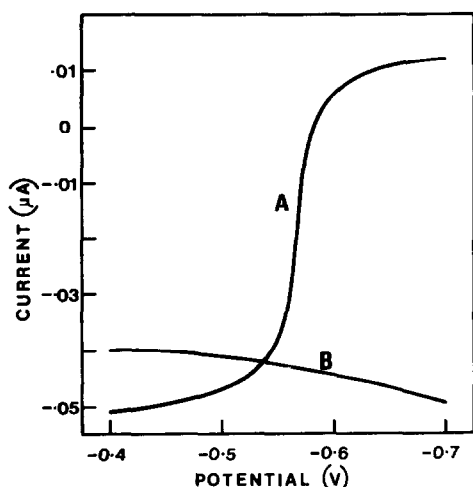


Fig. 1. Polarographic reduction wave of ferredoxin. A, 12 μ M ferredoxin in 0.1 M phosphate buffer, pH 7.0. B, 0.1 M phosphate buffer alone. The condenser-current compensation was 0.2 μ A/V.

versus the hydrogen electrode. This value is more positive than published data on the redox potential of *C. pasteurianum* ferredoxin. Thus, Tagawa and Arnon [4] first reported a potential of -413 mV at pH 7.1, determined colorimetrically with H_2 /hydrogenase, while in a more recent study [5] they redetermined this to be -385 to -390 mV. By a similar method Sobel and Lovenberg [6] reported a reduction potential of -400 to -410 mV at pH 7. On the other hand, Eisenstein and Wang [7] found the reduction potentials for the two electrons taken up by clostridial ferredoxin to be distinct, and reported values of -367 mV and -398 mV at pH 7.4. We are at present unable to account for the difference between the polarographic half-wave potential and the redox potentials determined by other procedures, though adsorption of the ferredoxin onto the mercury drop surface may be a contributory factor.

We have also observed a wave with wheat ferredoxin at about -620 mV (versus the S.C.E.), i.e. at a lower potential than the clostridial ferredoxin. This is consistent with the observations of Tagawa and Arnon [5] that plant ferredoxin has a lower reduction potential than clostridial.

There appears to be some disagreement over the pH dependence of the ferredoxin reduction potential.

Table 1
Dependence of the ferredoxin half-wave potential on pH.

pH	Half-wave potential (versus S.C.E.) mV
6.1	-530
6.6	-555
7.0	-570
7.4	-577
7.6	-578
8.0	-585
8.5	-585
9.2	-595

Tagawa and Arnon [5] found the potential to be independent of pH in the range investigated (pH 6.13–7.41) while Sobel and Lovenberg [6] found the potential to vary linearly over the range pH 5.5–9.0 with a slope of -45 mV/pH unit. We have investigated the dependence of the polarographic half-wave potential on pH (table 1) and our results indicate an intermediate situation. Increasing pH does move the half-wave potential to more negative values, but much less so than the reduction potential changes found by Sobel and Lovenberg [6]. We have found only a 65 mV change between pH 6.1 and 9.2.

The height of the ferredoxin wave was found to be dependent on concentration, but reached a limiting value at about 5μ M, depending on the characteristics of the particular dropping mercury electrode used. The concentration at which the wave height levelled off corresponded approximately with that producing immiscible drops from the electrode, a situation similar to that encountered with other proteins [3]. This limiting wave height probably arises from saturation of the mercury drop surface with a layer of protein molecules, permitting no further increase in electrolysis rate.

We have attempted to identify the reduction process giving rise to the polarographic wave with the physiological reduction by reducing ferredoxin with H_2 /hydrogenase [4] and examining the effect on the polarographic wave. The hydrogenase was a crude extract of *C. pasteurianum* [8] with an activity

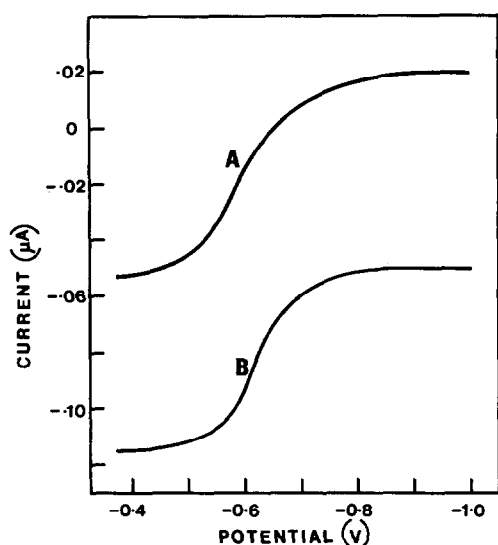


Fig. 2. Polarographic waves of oxidised and reduced ferredoxin. A, cathodic wave of 12 μ M ferredoxin in 0.1 M borate, pH 9.2. B, anodic wave produced after electroreduction at -1.5 V for 30 min.

of 1.5 μ mole H_2 liberated per min per mg protein when assayed in 50 mM Tris-HCl, pH 8.2, with 25 mM dithionite and 250 μ g methyl viologen per ml [9]. This preparation was stored under nitrogen. A solution of ferredoxin (12 μ M) in 50 mM Tris-HCl, pH 8.2, was degassed with nitrogen and a polarogram recorded. Five mg of hydrogenase preparation were added and the ferredoxin wave was essentially unchanged. The stream of nitrogen was then replaced by hydrogen and after 10 min a polarogram was recorded. The ferredoxin wave was virtually completely abolished. This result suggests that the reduction process underlying the polarographic wave of ferredoxin is the same as that involved in the physiological electron transfer.

Studies by one of us [3] on the polarography of disulphide-containing proteins showed that polarographic activity at the dropping mercury electrode was accompanied by electroreducibility at a stirred mercury pool cathode. We therefore investigated such electroreduction of ferredoxin. Using the method previously described [3] we electrolysed a solution of ferredoxin in 50 mM Tris-HCl, pH 8, at -1.5 V. Within 10–15 min we observed a marked

loss of the characteristic yellow-brown colour, indicating reduction of the ferredoxin. Electrolysis was continued until no further loss of colour could be discerned and was then stopped. On exposure of the solution to air for a few moments the original colour was rapidly regained, indicating oxidation of the reduced ferredoxin back to its original form.

We also observed that addition of the reagent 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) to electroreduced ferredoxin produced the yellow colour indicative of the presence of thiol groups, whereas no reaction occurred with oxidised ferredoxin. This shows that electroreduction has led to the formation of thiol groups not present, or not available for reaction, in the oxidised ferredoxin. Malkin and Rabinowitz [10] also observed a reaction between DTNB and ferredoxin reduced with borohydride in 8 M urea, but not with oxidised ferredoxin.

After this demonstration of the feasibility of electroreduction of ferredoxin we carried out similar experiments using the modified apparatus described in the Experimental section above. In this way we were able to examine the effect of electroreduction on the polarographic wave of ferredoxin. A solution of ferredoxin in buffer was first degassed with nitrogen and a polarogram recorded. The ferredoxin was then electroreduced at the mercury pool cathode at a potential of -1.5 V (versus the S.C.E.). After 30 min when there appeared to be no further loss of colour, a polarogram was again recorded. Typical results are shown in fig. 2. The cathodic wave produced by oxidised ferredoxin was replaced by an anodic wave, of similar half-wave potential, given by the reduced ferredoxin. On opening the cell to the atmosphere the ferredoxin was reoxidised, the colour restored, and a subsequent polarogram showed the regain of a cathodic reduction wave.

In conclusion we have demonstrated that ferredoxin from *C. pasteurianum* is polarographically active at the dropping mercury electrode and that the polarographic reduction is identifiable with the biological reduction process. Polarography thus provides an additional tool for the study of this important electron transfer protein and may permit investigations not accessible by other techniques. In addition we have shown that electrolysis is an effective method of reducing ferredoxin. The fact that no chemical or biological reducing agents are

introduced, and therefore that no excess reductant can be present, may, for particular studies, represent an advantage of this method for reducing ferredoxin.

Acknowledgement

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